An Evaluation of a Battery of Functional and Structural Tests as Predictors of Likely Risk of Progression of Age-Related Macular Degeneration

D. Grant Robinson,[1] Tom H. Margrain,[1] Clare Bailey,[2] and Alison M. Binns[3]

[1]School of Optometry and Vision Sciences, Cardiff University, Cardiff, United Kingdom
[2]Bristol Eye Hospital, Lower Maudlin Street, Bristol, United Kingdom
[3]Division of Optometry and Visual Sciences, School of Health Sciences, City, University of London, London, United Kingdom

Correspondence: Tom H. Margrain, Cardiff Centre for Vision Sciences, College of Biomedical and Life Sciences, Maindy Road, Cardiff University, Cardiff CF24 4HQ, UK; margrainth@cardiff.ac.uk.

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Purpose. To evaluate the ability of visual function and structural tests to identify the likely risk of progression from early/intermediate to advanced AMD, using the Age-Related Eye Disease Study (AREDS) simplified scale as a surrogate for risk of progression. The secondary aim was to determine the relationship between disease severity grade and the observed functional and structural deficits.

Methods. A total of 100 participants whose AMD status varied from early to advanced were recruited. Visual function was assessed using cone dark adaptation, 14 Hz flicker and chromatic threshold tests and retinal structure was assessed by measuring drusen volume and macular thickness. The predictive value of the tests was estimated using ordinal regression analysis. Group comparisons were assessed using analysis of covariance.

Results. Change in cone dark adaptation (cone τ) and yellow-blue (YB) chromatic sensitivity were independent predictors for AMD progression risk (cone τ, pseudo $R^2 = 0.35$, $P < 0.001$; YB chromatic threshold, pseudo $R^2 = 0.16$, $P < 0.001$). The only structural predictor was foveal thickness ($R^2 = 0.05$, $P = 0.047$). Chromatic sensitivity and cone dark adaptation were also the best functional tests at distinguishing between severity groups. Drusen characteristics clearly differentiated between participants with early and advanced disease, but were not able to differentiate between those with early AMD and controls. Mean differences in retinal thickness existed between severity groups at the foveal ($P = 0.040$) and inner ($P = 0.001$) subfields.

Conclusions. This study indicates that cone τ, YB chromatic threshold and foveal thickness are independent predictors of likely risk of AMD progression.

Keywords: age-related macular degeneration, visual function, progression

AMD is the leading cause of sight loss in the developed world.[1,2] Currently, treatment is available only for those with neovascular AMD (nAMD). Therefore, the development of effective treatments for early AMD, intermediate AMD, and geographic atrophy (GA) is paramount. In designing clinical trials of interventions aimed at preventing disease progression, identifying those individuals at greatest risk will facilitate an efficient study design.

Longitudinal data from the Age-Related Eye Disease Study (AREDS) led to the development of grading scales which enable the estimation of the 5-year risk of progression to advanced AMD.[3,4] The AREDS Simplified Severity Scale can be used to estimate the risk of progression to advanced AMD by summing risk factors which include the presence of large drusen, pigmentary abnormalities, or advanced AMD in one eye.[3] The lowest and highest risk groups (0 and 4 risk factors) were estimated to exhibit a 0.5% and 50% chance, respectively, of progression to advanced AMD over a 5-year period.

Another approach to predicting risk of disease progression lies in the evaluation of visual function. Parameters such as rod and cone dark adaptation,[5–10] flicker threshold,[5,11,12] and chromatic sensitivity[13–15] have been shown to be promising biomarkers for AMD progression. The relationship between these functional parameters and measurements of retinal structure associated with AMD progression, such as drusen volume and retinal thickness, has not yet been established.[16–19] It may be that structural and functional measures provide complementary information about disease status which may facilitate improved clinical monitoring.

The primary aim of this study was to evaluate the ability of a battery of visual function tests to identify the likely 5-year risk of progression from early to advanced AMD, using the AREDS simplified severity scale[3] as a surrogate for risk of progression. The secondary aim was to determine the relationship between disease severity and the observed functional and structural deficits.

Methods

Participants

This cross-sectional study took place at the Bristol Eye Hospital, Clinical Research Unit from July 2014 to August 2016. All participants were aged between 55 and 88 years and had a best

corrected visual acuity (BCVA) in the test eye of 0.3 logMAR or better. Of those participating, 55% were female. Exclusion criteria included bilateral advanced AMD, ocular pathology other than macular disease, significant cataracts (above grade 3 on any LOCS III criterion),[20] systemic disease or history of medication known to affect visual function, and cognitive impairment as determined using an abridged Mini Mental State Examination.[21]

We recruited 100 participants to the study. Data from 60 individuals were collected as baseline for the ALight Clinical Trial (ISCTRN number 82148651).[22] These participants exhibited early/intermediate AMD in one eye and nAMD in their fellow eye, according to the Beckman initiative grading scale,[23] and were recruited directly from the Bristol Eye Hospital Medical Retina Clinic. The remaining 40 participants had no AMD or early/intermediate AMD only, and were recruited from a list of research volunteers at Cardiff University Eye Clinic, clinic waiting rooms at Bristol Eye Hospital and from among the friends and family of participants. Those classified as having no AMD (group 0/'controls') had no soft drusen or focal pigmentary changes in the macula of either eye but could have "drupelets" (i.e., drusen $\leq$63 μm). This comprised the "no apparent aging changes" and "normal aging changes" stages of the Beckman initiative grading scale.[23] In those with unilateral nAMD, the nonneovascular eye was selected for testing. In cases where fundus status was the same between eyes, the eye with best visual acuity was the test eye, or the right eye in the case of equal acuities.

All participants provided informed written consent prior to participation. The study was approved by the NHS Health Research Authority NRES Committee North West—Greater Manchester South (13/NW/0609), and all procedures adhered to the tenets of the Declaration of Helsinki.

Experimental Procedure

All participants underwent a standardized baseline clinical examination. This included measurement of refractive error, assessment of BCVA (letter by letter logMAR score using 4-meter Early Treatment of Diabetic Retinopathy Study [ETDRS] chart), slit-lamp biomicroscopy examination of the anterior and posterior segment, and a semistructured interview regarding ocular history, medical history, and medication. Any participants who reported a history of congenital color vision deficiency were excluded from the color vision analysis. Pupillary dilation was achieved using 1% tropicamide prior to visual function testing.

The same room was used for all visual function tests. Each participant was seated 140 cm from a high-resolution LED monitor (NEC MultiSync PA24/W). The luminance output of the monitor was γ corrected in accordance with the method described by Metha et al.[24] When measuring dark adaptation and flicker thresholds, the stimulus was driven by an 8-bit graphics board (Geforce 9; nVIDIA, San Jose, CA, USA) under software control (MATLAB, R2009a, The MathWorks Inc., Natick, MA, USA). Prior to the collection of color discrimination thresholds using the color assessment and diagnosis (CAD) test the monitor was calibrated using the bespoke LUMCAL program as supplied by City Occupational Ltd (London, UK).

Distance refractive correction was worn if required and the nontest eye was occluded. Test order was randomized and all were performed on one visit. Participants were given short breaks between tests to prevent fatigue. When necessary, participants reattended to repeat a psychophysical test if completion was not possible at their initial visit. All repeat measurements were recorded within 4 weeks of initial assessment. Retinal images were obtained following visual function testing.

Chromatic Sensitivity Measurement

Red-green (RG) and yellow-blue (YB) chromatic thresholds were measured using the CAD test, (Version 2.2.4., City Occupational Ltd.), according to the method described by O'Neill Biba et al.[15] Briefly, the stimulus comprised a color-defined checkerboard of 5 × 5 squares (total 1.1° diameter) which, over a period of 600 ms, moved diagonally across an achromatic background of dynamic luminance noise (chromaticity coordinates 0.305, 0.323; mean luminance 26 cd/m^2) in one of four possible directions. The CAD test measures detection thresholds in 16 hue directions in the CIE (1931) chromaticity chart. The test employs an efficient, 4-alternative forced choice procedure with 16 interleaved staircases with variable step sizes and 12 reversals. The threshold is based on the average of the last six reversals. The thresholds are calculated as the linear distance on the chromaticity diagram between the target and the background chromaticity at threshold. The CAD unit is defined as the median color signal strength needed by young, healthy, normal trichromats (based on 330 young subjects). It corresponds to ~0.4% L and 0.8% M cone contrasts.[25] The participant was instructed to press the button on a handheld keypad that corresponded with the perceived direction of movement of the stimulus. Once a firm understanding of test requirements had been demonstrated (100% correct response rate in a training program) the participant progressed onto the full threshold measurement program.

Dark Adaptation Measurement

The dark adaptation parameter measured was cone τ (time taken for sensitivity to recover to 1-1/e of the prebleach value). Cone dark adaptation thresholds were measured using a "3 down, 1 up" staircase procedure with a 0.2-second stimulus presentation time and a 0.6-second response window, according to the protocol described elsewhere.[9,26–28] In brief, thresholds were recorded in response to a 2° radius, solid yellow circular stimulus (chromaticity coordinates, 0.429, 0.413), centered on the fovea. Data collection was preceded by a short (no bleach) familiarization session, and the subject was permitted to proceed only if they were able to complete the training without any erroneous responses. A handheld bleaching source consisting of a "white" LED overlaid with a diffusing (LEE Filters 216 "white diffusion") and amber filter (LEE Filters HT015 "deep straw") was used to deliver a 120-second photopigment bleach of retinal illuminance 5.20 log phot Td.s^{-1} (bleaching approximately 85% of cone[29] and 74% of rod[30] pigment), to a retinal area subtending 12°. The bleach source was calibrated using a photometer (LS-110; Konica Minolta, Osaka, Japan). Upon termination of the bleach, dark adaptation was monitored continuously for 25 minutes.

Flicker Threshold Measurement

Temporal sensitivity was measured using a well-established QUEST Bayesian adaptive procedure,[31–33] according to the protocol described by McKeague et al.[22] The QUEST procedure was implemented using routines available within the computing environment (MATLAB Psychophysics Toolbox, R2009a; The MathWorks Inc.) to drive a go/no-go adaptive staircase. In brief, the trial stimulus was a 4° Gaussian blob (chromaticity coordinates, 0.305, 0.323; temporal frequency 14 Hz) surrounded by a white circle to aid fixation. The subject pressed a button on a handheld keypad as soon as they perceived the stimulus. A familiarization test of 10 trials was performed before data collection and formed the starting point for the final threshold estimate. The full trial of 40 presentations

Table 1. Characteristics of Each Graded AMD Severity Group

Group	Participants	Age	Visual Acuity (ETDRS) Study Eye		Visual Acuity (ETDRS) Fellow Eye	
AREDS Grade	*N* (% female)	Mean (±SD)	Mean (±SD)	Age-Adjusted Mean (±SD)	Mean (±SD)	Age-Adjusted Mean (±SD)
0	19 (52%)	69.58 (±8.73)	−0.01 (±0.07)	−0.02 (±0.07)	0.08 (±0.12)	0.05 (±0.15)
1	21 (66%)	74.33 (±6.44)	0.03 (±0.14)	0.03 (±0.14)	0.20 (±0.28)	0.19 (±0.28)
2	18 (44%)	76.56 (±8.29)	0.06 (±0.15)	0.06 (±0.16)	0.30 (±0.31)	0.31 (±0.33)
3	23 (56%)	78.65 (±6.52)	0.08 (±0.12)	0.08 (±0.12)	0.45 (±0.28)	0.46 (±0.28)
4	19 (58%)	77.74 (±5.37)	0.11 (±0.11)	0.11 (±0.11)	0.45 (±0.35)	0.47 (±0.35)

Raw and age-adjusted means are provided for visual acuity measurements. Where mean values are given, brackets denote the SD.

commenced when the participant achieved two successive measurements in the familiarization test within 1 standard deviation of each other with no more than 1 false positive.

Examination of Structural Outcome Measures

Fundus photographs (30° diameter centered on the fovea) were taken using a 3D-OCT device (Topcon 3D-OCT 2000; Topcon Medical Systems Inc., Oakland, NJ, USA) and digitally stored. The fundus images were graded according to the AREDS Simplified Scale[3] by two independent graders. Where disagreement occurred, the results were adjudicated by the chief investigator (AB).

The acquisition of SD-OCT images was performed using a SD-OCT device (Zeiss Cirrus SD-OCT 4000; Carl Zeiss Meditec, Inc., Dublin, CA, USA). Five macular cube scans (200 × 200 A scans) covering a retinal area of 6×6 mm^2 were obtained for each participant. All scans were obtained by experienced operators. Following precedent, a signal strength of 7 or greater was required for all images.[16] Drusen volumes and areas within 3- and 5-mm circles (centered on the fovea) were estimated using analytical software (Cirrus Advanced RPE Analysis Software, Version 7.0.1, Carl Zeiss Meditec) and averaged across the five scans.

Retinal thickness was quantified using commercial software (OCT Explorer 4.0; Retinal Image Analysis Lab, Iowa Institute for Biomedical Imaging, Iowa City, IA, USA).[34,35] Of the five images taken per participant, the scan with the highest signal strength was chosen for automated retinal layer segmentation. Total retinal thickness (from the inner limiting membrane to the outer boundary of the RPE) was averaged for the foveal subfield (1 mm diameter) and the inner (3 mm diameter) and outer (6 mm diameter) rings of a standard ETDRS grid.

Statistical Analysis

All statistical analysis was performed using statistical software (SPSS Statistics 20.0, SPSS Statistics for Windows, R2011; IBM Corp., Armonk, NY, USA). To determine whether there were significant differences in age between groups a 1-way ANOVA was conducted. The nominal level of statistical significance was set at $\alpha = 0.05$. In order to remove the effect of age as a potentially confounding factor, linear regression analysis was used to model the relationship between each outcome measure and participant age within the control group. If the relationship was significant, the data for each participant were adjusted according to the linear regression equation to that of the mean age of the entire cohort (75.37 ± 7.07 SD). The drusen parameters were not age-adjusted as they were only associated with advancing disease. The Shapiro-Wilkes test was used to assess distributional assumptions.

The predictive value of the visual function tests to identify the likely risk of progression of AMD (using the AREDS Simplified Severity scale as a surrogate for 5-year risk of progression) was estimated using ordinal regression analysis. The sample size of 100 would allow an 8% difference in 5-year progression risk between controls and those with AMD to be detected at a probability level of 0.2 and power of 80%. Ordinal regression models identified as statistically significant ($P < 0.05$) were used to determine odds ratios (calculated as the exponential of each coefficient estimate) for individual and combinations of outcome measures. The assumption of multicollinearity was verified using the test of parallel lines and coefficient estimates were verified as significant ($P < 0.05$, based on Wald test statistic) prior to odds ratio calculation. Odds ratios equated to the odds of progressing up an AMD severity grade based on 1 unit increase of the outcome measure. The corresponding risk of 5-year progression to nAMD associated with each severity grade was designated as grade 0 (0.4%), grade 1 (3.1%), grade 2 (14.8%), grade 3 (35.4%), grade 4 (53.1%) in accordance with the AREDS Simplified Severity Scale.[3]

In order to determine the relationship between disease severity and each outcome measure, comparisons between severity groups were conducted using the 1-way ANOVA test (for normally distributed data) or the Kruskal-Wallis with pairwise Mann-Whitney *U*-test post-hoc comparisons (for nonnormally distributed data). In order to account for multiple comparisons, *P*-values were adjusted in accordance with the Holm-Bonferroni method.[36] Standard and multiple linear regression analysis were conducted to explore the relationship between the structural (predictor variable) and functional (outcome variable) outcomes. Regression assumptions were tested in accordance with methods described by Altman.[36]

Results

One hundred participants were recruited, with AMD severity distributed from grade 0 to 4 (mean age 75.37 ± 7.07 SD; 55% female). In those with unilateral nAMD, the number of prior intravitreal injections ranged from 3 to over 28. The mean age of the grade 0 group (69.58 ± 8.73 years) was significantly lower than grades 2 (76.56 ± 8.29 years; $P = 0.037$), 3 (78.65 ± 6.52 years; $P = 0.001$) and 4 (77.74 ± 5.37 years; $P = 0.006$). Mean refractive error did not differ significantly between groups (mean sphere grade 0 = –0.25 DS, grade 1 = +1.00 DS, grade 2 = –0.25 DS, grade 3 = +0.50 DS, grade 4 = +0.50 DS; 1-way ANOVA *P*=0.209). See Table 1 for details.

Chromatic thresholds, cone τ and 14-Hz flicker thresholds were recorded for all participants. There was no significant difference in test results for any functional test depending on the order in which they were conducted (1-way ANOVA CAD, $P = 0.102$; flicker, $P = 0.573$; DA, $P = 0.528$). One participant was removed from the RG chromatic sensitivity analysis due to a self-reported life-long congenital protan-type defect. Overall, 85% of male and 83% of female participants failed the CAD test in the study eye (i.e., fell outside the normal reference range for color discrimination). This proportion did not differ between sexes (χ^2 test; $P = 0.824$). Significant relationships

Table 2. Independent Predictors of AMD Risk of Progression

	P	Pseudo R^2	β Coefficient Estimate	Odds Ratio	95% Confidence Interval
Cone τ	<0.001	0.35	3.352	28.56	2.022 to 4.681
YB threshold	<0.001	0.16	1.408	4.09	0.607 to 2.209
Foveal subfield thickness	0.047	0.05	−0.014	1.00	−0.029 to 0.000

Data shown are for significant predictors of risk of progression as determined by the AREDS simplified grading scale.[9]

with age were found in control participants for visual acuity, $P = 0.02$; RG chromatic threshold, $P = 0.02$; YB chromatic threshold, $P = 0.05$; cone τ, $P = 0.01$ and 14-Hz flicker threshold, $P = 0.05$. The functional data were therefore all corrected for the effect of age (Supplementary Table S1). There was no significant relationship between age and retinal thickness (foveal subfield, $P = 0.166$; inner subfield, $P = 0.436$; outer subfield, $P = 0.571$), hence structural parameters were not age adjusted.

Functional and Structural Outcome Measures as Predictors of Risk of Progression

Data for significant predictors of increased risk of AMD progression, according to the AREDS simplified grading scale, are shown in Table 2. Where data were not normally distributed log transformation was performed prior to ordinal regression. When tested independently, cone τ (pseudo R^2 0.35, $P < 0.001$) and YB chromatic sensitivity (pseudo R^2 0.16, $P < 0.001$) were found to be independent predictors of increased risk of AMD progression according to the AREDS simplified severity scale. With every log minute increase in cone τ and every 1-unit increase in the CAD threshold, the odds of moving up a severity grade were increased by 28.56 and 4.09 times, respectively. The individual predictive capacity of cone τ and YB chromatic sensitivity was further heightened via their combined use (pseudo R^2 0.39, $P < 0.01$). RG chromatic sensitivity and flicker threshold were rejected as significant predictors by all ordinal regression models. Foveal thickness was the only significant structural predictor ($P = 0.047$). However, this structural parameter explained less of the variance (pseudo R^2 0.05) than cone τ and was rejected by all multivariate models.

Retinal Function and Graded Disease Severity

Age-adjusted group means for each functional outcome measure for each severity grade are provided graphically in Figure 1. Data were normally distributed within each severity grade (Shapiro-Wilkes, $P > 0.05$) for the functional outcome measures (following log transformation) and for measurements of retinal thickness. The remaining structural outcome measures were not normally distributed even after log transformation, hence nonparametric tests were used to evaluate these data. The mean RG chromatic threshold was significantly lower in the grade 0 group than grades 2 ($P < 0.001$), 3 ($P = 0.001$), and 4 (Fig. 1B, $P < 0.001$). When assessing YB chromatic threshold, the mean for grade 4 was significantly higher than all other groups (Fig. 1C, grade 0, $P < 0.001$; grade 1, $P < 0.001$; grade 3, $P < 0.021$), except grade 2 ($P = 0.683$). Unlike RG thresholds, there were no significant differences found between those graded 1 through 3. Mean cone τ values were significantly lower in grade 0 than grades 3 ($P = 0.033$) and 4 ($P < 0.001$), and in grade 1 when compared to all subsequent grades (Fig. 1E, grade 2, $P = 0.032$; grade 3, $P = 0.001$; grade 4, $P < 0.001$). There was no difference in mean 14-Hz flicker threshold values between grade 0 and those graded 1 through 3 (Fig. 1D). The average flicker threshold value was significantly higher for those with the highest graded AMD severity when compared to all other grades (grade 0, $P < 0.001$; grade 1, $P < 0.001$; grade 2, $P = 0.015$; grade, $P < 0.001$).

Retinal Structure and Graded Disease Severity

Drusen area, volume and retinal thickness values were obtained for all participants. Median drusen volume and area measurements were found to be nil for those graded 0 to 2 (Supplementary Table S2). Those graded ≥3 were found to have a significantly larger drusen volume and area when compared to those graded ≤2 (Fig. 2).

Mean differences in retinal thickness (see Supplementary Table S3) existed between severity groups at the foveal ($P = 0.040$) and inner ($P = 0.001$) subfields. The mean foveal subfield was found to be significantly thicker in those graded 0 when compared to those graded 4 ($P = 0.04$; Fig. 3A). No significant differences in thickness were found between grades 0 and 3. Similarly, grade 4 subjects had a significantly thinner mean inner-subfield zone than those graded 0 ($P = 0.001$), 1 ($P = 0.015$), and 3 ($P = 0.018$; Fig. 3B).

Discussion

Using the AREDS Simplified scale as a surrogate, cone dark adaptation was found to be the best predictor of risk of AMD progression. Of the remaining three functional tests, YB chromatic sensitivity had the greatest value as a marker of AMD progression risk. Chromatic sensitivity and cone dark adaptation were also best at distinguishing between AMD severity grades and showed the closest relationship to retinal structural changes.

These findings are consistent with Eisner et al.,[13] who identified foveal dark adaptation rate and low S-cone mediated sensitivity as the strongest functional predictors of nAMD development. The predictive value of dark adaptation is also supported by Owsley et al. who showed that those with a normal retinal appearance but delayed rod dark adaptation at baseline were twice as likely to have AMD in that eye when reassessed after 3 years.[6] Age-related macular degeneration and the rate limiting step in the dark adaptation process share the same anatomic locus, the RPE,[37] and it is for this reason that measures of dark adaptation are consistently associated with AMD, even at its earliest stages. Although previous studies have reported that flicker sensitivity is reduced in eyes that eventually develop GA or choroidal neovascularization[12,38] the current findings, like those of Eisner et al.[13] suggest that this test has less value as a predictor of disease onset and progression.

Cone adaptation was selected rather than rod adaptation as an outcome measure in this study primarily for pragmatic reasons. A valuable biomarker for disease progression should be evaluated within a clinically viable timeframe. Although recent studies have demonstrated that an individual can be classified as having "abnormal" rod adaptation in as little as 6.5 minutes,[39] it can take much longer to determine a precise rate of rod adaptation in people with AMD. In a study measuring

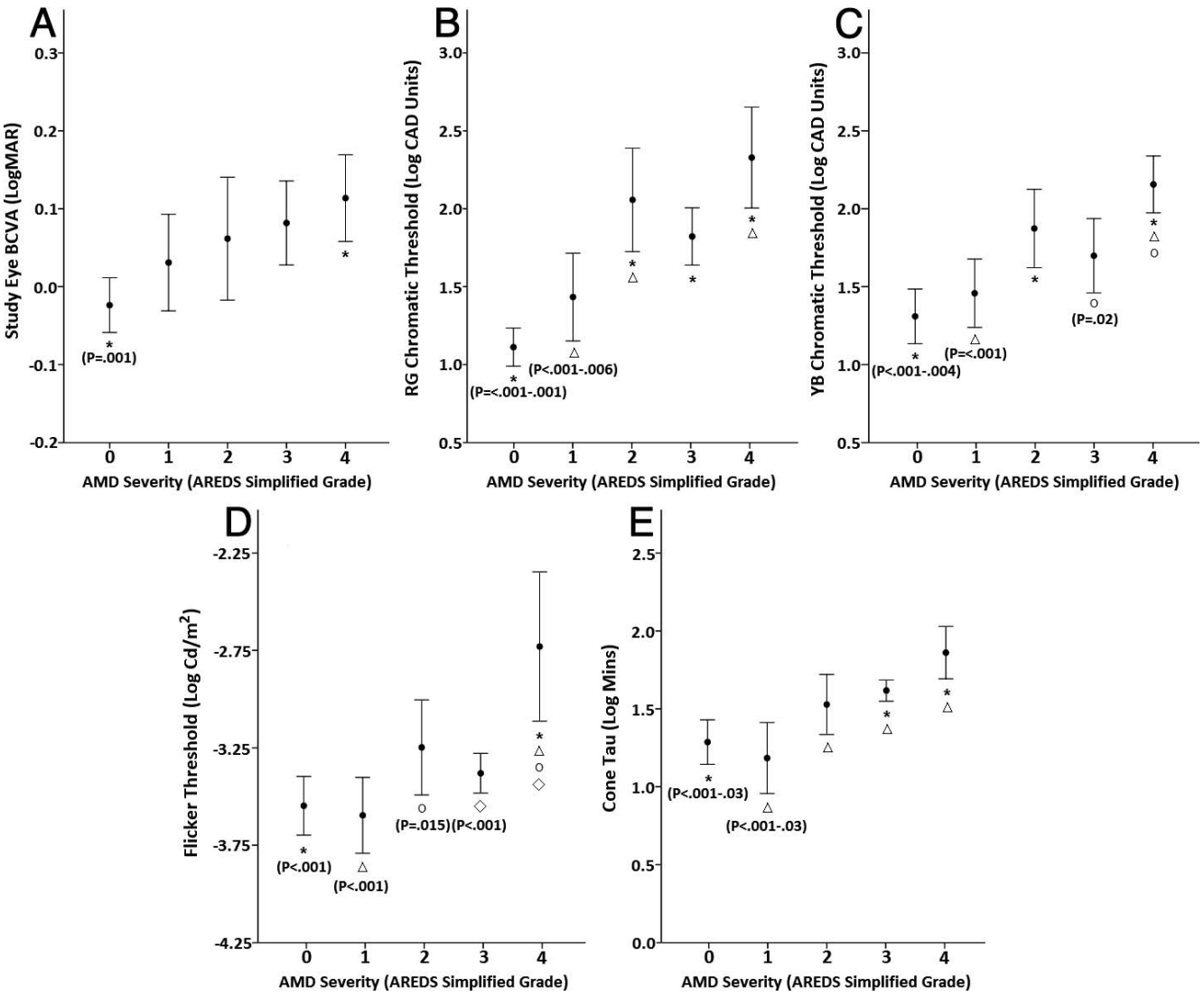

FIGURE 1. Graphs showing differences in age-adjusted means across AREDS grades for 5 functional measures: visual acuity (**A**), RG chromatic sensitivity (**B**), YB chromatic sensitivity (**C**), 14 Hz flicker threshold (**D**), and cone τ (**E**). Significant differences between groups are denoted by the four symbols used (*asterisk*, *triangle*, *circle*, and *diamond*). A symbol above a specified *P*-value denotes a significant difference between that severity grade and all others with the same symbol. When a significant difference exists between one grade and multiple others the range of *P* values is displayed. *Error bars* denote 95% confidence intervals.

the time taken to reach a criterion threshold on the second component of rod recovery in 30 eyes with intermediate AMD, recovery times ranged from 8.8 to 124.4 minutes.[40] Despite the proposed alternative mechanism for photopigment regeneration available to cones through the Müller cell pathway,[41,42] it appears that cone adaptation is also progressively affected by AMD disease progression.[5,8,9,26,43–46]

The poor predictive capacity of RG chromatic sensitivity in comparison to YB sensitivity is also consistent with previous evidence as based on the longitudinal study of 47 subjects with macular drusen reported by Holz et al.[47] The vulnerability of the S-cone pathway to retinal disease has been attributed to the physiology of S-cone receptors and their corresponding ganglion cells.[48–50] The limited dynamic response range of the S-cone receptors compared to L and M counterparts has been postulated as a factor resulting in their increased susceptibility to pathologic disturbance such as the hypoxia and inflammation implicated in AMD pathogenesis.[48] The loss could also be due to alterations in the postreceptoral retinal mechanisms.[51]

A good functional biomarker would also be able to monitor disease progression; hence, we also evaluated the ability of all tests to differentiate between AREDS severity grades. Although all vision function tests assessed in this study displayed potential to distinguish between controls and those in the highest AMD severity grading, chromatic sensitivity, and cone-dark adaptation were best at distinguishing between severity groups. These findings are broadly consistent with the findings of Dimitrov et al.[5] who reported that the functional biomarker with the best diagnostic capacity to distinguish those with early AMD ($n = 221$) from controls ($n = 109$) was cone recovery rate, followed by 14-Hz flicker threshold, blue color threshold, and red color threshold. In a second cross-sectional study Dimitrov et al.[43] reported that the adaptational tests of cone and rod recovery rate exhibited a rapid decrease in functional outcomes at the earliest stage of AMD, plateauing at a low level of function that remained stable despite more severe clinical changes. In comparison, 14-Hz flicker threshold and blue chromatic threshold decreased consistently as clinical grading increased in severity. Similarly, the present study found

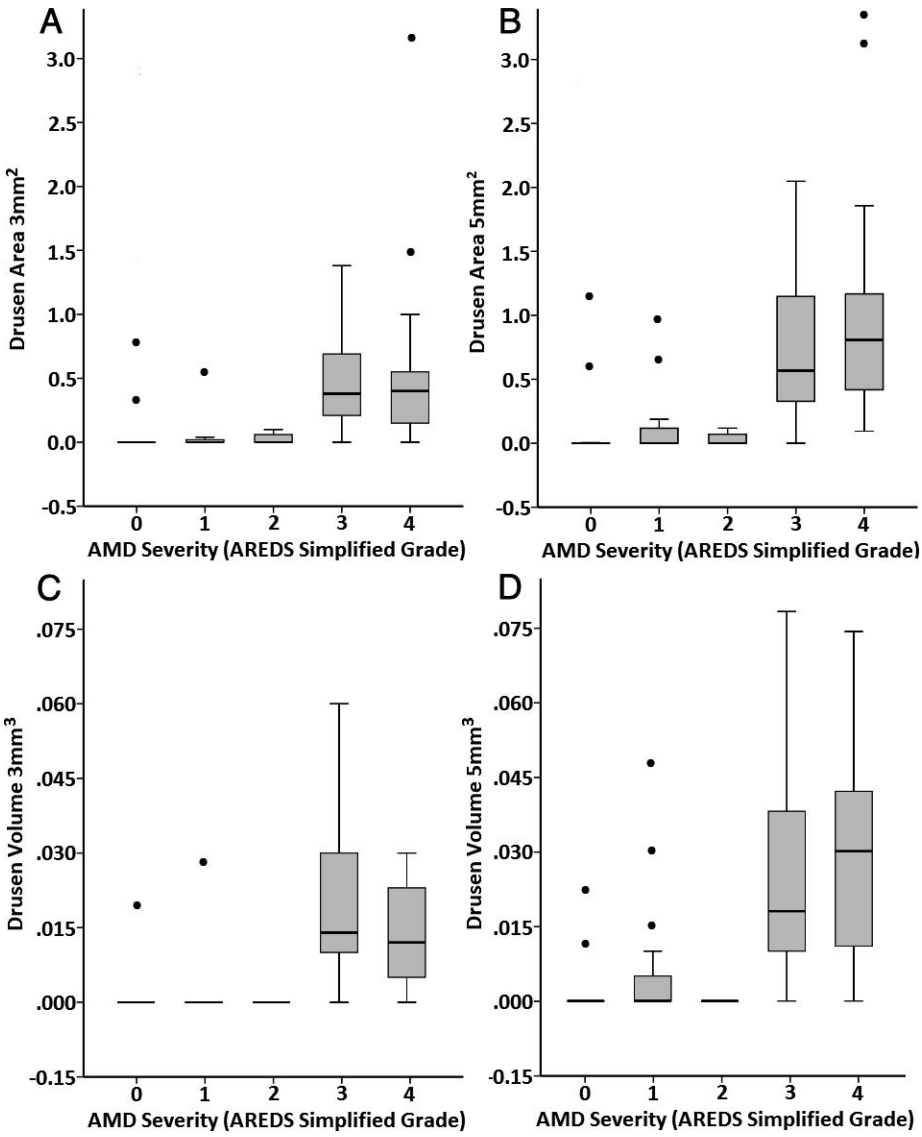

FIGURE 2. Boxplots for each structural outcome measure showing distribution of data (drusen area 3 mm^2 (**A**), drusen area 5 mm^2 (**B**), drusen volume 3 mm^2 (**C**), and drusen volume 5 mm^2 (**D**) across graded AMD severities. *Black circles* show outliers (calculated as 3 times above the interquartile range). One drusen volume outlier (GP15: 1.66/1.74 mm^3 is not shown as their data extends beyond axis boundaries). A negative *y*-axis origin has been chosen in order to aid visualization of the minimum value for all structural outcomes (0.00).

that cone τ was not useful in distinguishing between those within the highest severity gradings (grade 3 and 4); however, it was able to distinguish those with the earliest disease severities (grades 0 and 1) from those with more advanced clinical presentations, while chromatic sensitivity measurement was more useful in determining significant differences across the entire spectrum of graded severities. This suggests that the choice of functional biomarker in a clinical trial may depend on the stage of AMD being evaluated.

In this investigation, we chose to randomize test order. While this may have minimized the impact of order effects such as fatigue, we cannot rule out the possibility that tests subsequent to the measurement of cone dark adaptation may have been affected by the photopigment bleach. To mitigate this risk the study design ensured that a minimum of 35 minutes elapsed after bleach cessation before the onset of the next test. Even the individual with the most delayed adaptation (cone tau 13.6 minutes) would have recovered to 92% of their baseline cone sensitivity after this time. Furthermore, we note the absence of an order effect in the analysis.

Drusen volume was not identified as a predictor of risk of AMD progression despite the presence of large drusen being a risk factor according to the AREDS Simplified Severity Scale. This may reflect the relative insensitivity of the analysis software (Carl Zeiss Meditec) and imaging protocol to small elevations of the RPE. The elevation map generated by the software apparently permits measurement of drusen area and volume elevations >20 μm[52]; however, it may be that the onboard drusen analysis software (Carl Zeiss Meditec) used in this study was not sensitive enough to identify the small RPE deformations seen in some fundus images. Despite this, the drusen characteristics measured allowed clear differentiation between participants with early and advanced disease grades. It is possible that other drusen measures, such as drusen number, location and type, and effect on overlying structure (such the integrity of the IS/OS junction) might show greater predictive sensitivity. For example, the AREDS simplified scale

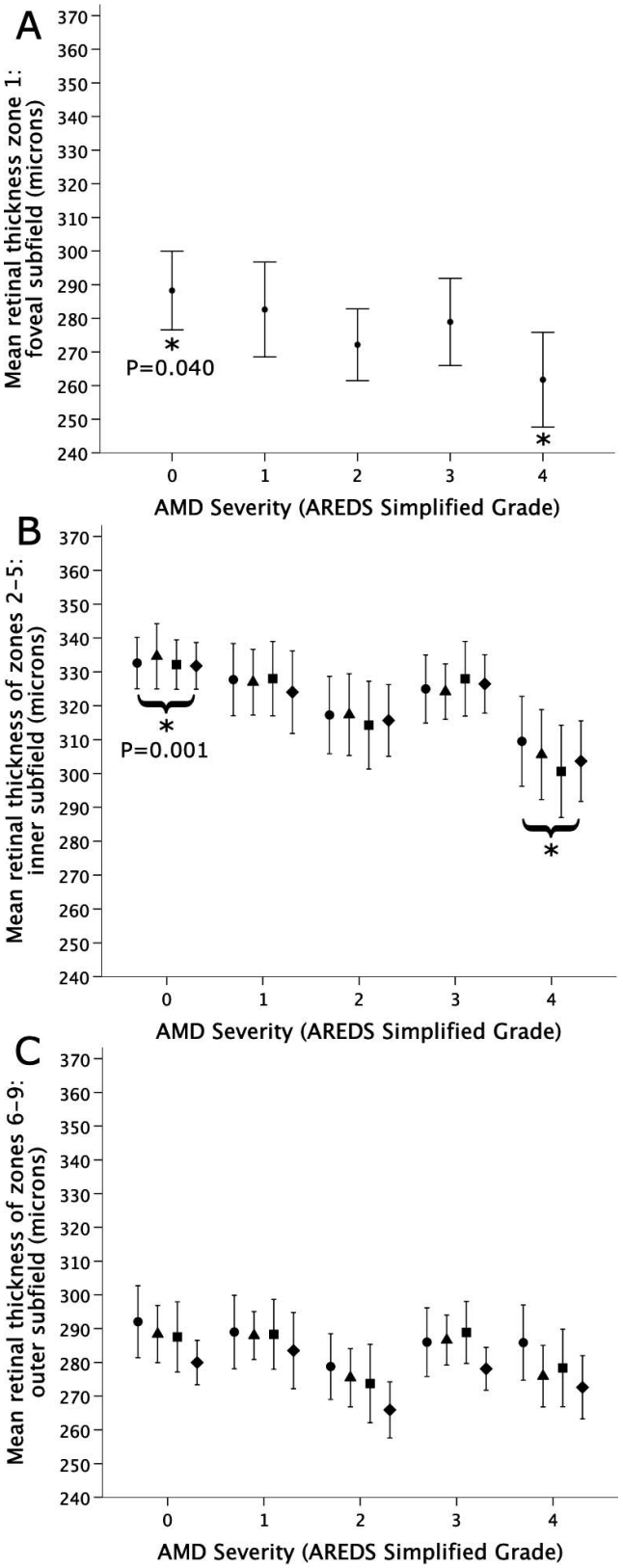

FIGURE 3. Graphs showing differences in means across AREDS grades for all 9 zones of the ETDRS grid. Panels show retinal thickness data for foveal (**A**), inner (**B**), and outer (**C**) subfields. For data relating to the inner and outer subfields, each ETDRS zone is denoted by a different symbol; *circle* (zone 2 plot B, zone 6 plot C), *triangle* (zone 3 plot B, zone 7 plot C), *square* (zone 4 plot B, zone 8 plot C), and *diamond* (zone 5 plot B, zone 9 plot C). Significant differences between thickness values (averaged across subfield) of AMD severity groups are denoted by an *asterisk* and *P* value. *Error bars* denote 95% confidence intervals.

is based on the premise that the presence of large drusen is indicative of an increased risk of progression.[3] Of particular note is that fact that the drusen volume software employed in this study, based on RPE deformation, is also unlikely to detect the presence of subretinal drusenoid deposits (SDD, also known as reticular pseudodrusen), which are located between the retina and the RPE. The prevalence of SDD in people with early/intermediate AMD may be as high as 62%, depending on the absence/presence of fundus pigmentary changes.[53,54] This is significant as the presence of SDD has been associated with a significantly increased risk of progression to advanced AMD, and is also linked to increased delays in rates of dark adaptation and elevated chromatic thresholds.[55–57] However, current software does not allow these drusen and SDD features to be automatically assessed in OCT images. Furthermore, the evaluation of SDD presence/absence is more reliable when multimodal imaging is employed, including infrared imaging.[58] These factors mean that the clinical application of these measures at present is likely to be limited in scope. Retinal thickness measurements also showed limited ability to differentiate between stages of disease severity, but there was evidence of a thinning of the retina in the foveal and inner subfield regions in those with the highest AMD grade. A number of studies have evaluated retinal thickness in eyes with early and intermediate AMD.[19,59–61] Wood et al.[19] reported a reduction of retinal thickness at the fovea, and at a number of extrafoveal points in those with early AMD ($n = 16$) compared to age-matched controls ($n = 16$). The other studies documented focal thinning localized to drusen location but no evidence of a generalized reduction across the macular region.[59–61]

One limitation of this study was the reliance on the AREDS simplified severity scale[3] as a surrogate for risk of progression to advanced AMD. Although the scale is based on robust clinical trial data, the predicted progression risk is a marker for AMD progression and not actual risk. Longitudinal studies are needed to verify these findings. We chose to use cone rather than rod dark adaptation hence, our findings say nothing about the relative value of these two measures. Rather, our findings provide additional support for the use of dark adaptation metrics in clinical trials of AMD. In addition, multiple tests of significance were conducted to discern relationships between outcome measures and AMD progression. Although Bonferroni correction was used to reduce the risk of type I error, repeated testing does increase the risk that significant results arose by chance alone.

A further potential limitation relates to the dilation of the pupil before color vision testing. Light entering the edge of the pupil is perceived to have a slightly different color appearance when compared to that entering the center of the pupil.[62] However, we are not aware of any evidence that this would result in false positive color vision deficiency result. Pupil diameter will also have influenced retinal illuminance, however, the background illuminance of the CAD test is designed such that RG and YB thresholds are on a plateau where an increase or decrease in retinal illuminance of less than fourfold will have minimal effect on measured thresholds.[25] An additional issue relates to a modest amount of refractive blur, amounting to 0.75 diopters (D), induced by testing at 1.4 m. However, the colored target does not have recognizable spatial features and subtends 30 minutes arc,

corresponding to a required visual acuity of 6/180. For these reasons, the impact of refractive blur and pupillary dilation on the threshold measured is likely to have been minimal. Another limitation related to color vision testing is that it was not possible to distinguish between congenital and acquired color vision deficits in the analysis as only one eye was tested using the CAD procedure from each participant. To mitigate this, all participants were asked to report if they had a history of color vision abnormality. Anyone who thought that they may have a congenital color vision defect was removed from this part of the analysis. It is possible that some participants had undiagnosed congenital color vision deficiency, and so were included in the analysis. Given the X-linked recessive genetic transmission of RG congenital color vison deficiencies,[63] it would be expected that more males than females would have a color vision defect according to the CAD test in this study, were there to be a significant proportion of people with undiagnosed congenital color vision deficiency. However, there was no significant difference in the prevalence of color vision defects according to sex, suggesting that congenital deficiencies did not impact significantly on the results.

The final limitation of this study relates to the retinal thickness analysis. In common with most onboard software on commercial devices, a single lateral scaling value was used in our analysis to determine the limits of the ETDRS grid when assessing average retinal thickness. However, it has been reported that individual differences in axial length may influence the lateral magnification of the OCT image by up to 30%.[64] Differences in retinal magnification between individuals will mean that the actual image area covered by the ETDRS grid will have varied between participants in this study, which may have introduced a confounding factor into the analysis.[65,66] However, the refractive errors were on the whole modest (mean sphere ranged from −0.25 to +1.00 DS across groups), and did not differ significantly between severity groups. This suggests that axial lengths were also comparable between groups. Thus, as this study presents an analysis of mean results, the failure to correct for axial length in this analysis only introduces a small amount of additional variance, not a systematic problem.

The study is strengthened by the relatively large sample size, of which 60% of participants were recruited from a clinical trial cohort.[22] Also, the robust outcome measures tested each relate to a body of evidence supporting their use as effective AMD biomarkers.

In conclusion, the findings of this study suggest that cone dark adaptation and YB color discrimination are likely to be the most useful biomarkers. Furthermore, the identification of two different visual function testing modalities as independent predictors of AMD progression risk implies that cone τ and YB chromatic sensitivity may provide different information about the underlying pathological processes. However, this study evaluated ability to predict risk of progression based on the fundus appearance graded using the AREDS simplified scale.[3] While this approach highlighted promising candidate functional biomarkers, there is a need for a longitudinal evaluation of these functional measures over a period of years in order to quantify definitively the risk of progression associated with a change in these parameters.

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